

iGEM TU/e 2014

Biomedical Engineering

Eindhoven University of Technology

Room: Ceres 0.04

Den Dolech 2, 5612 AZ Eindhoven

The Netherlands

Tel. no. +31 50 247 55 59

2014.igem.org/Team:TU_Eindhoven

FACS - Antibody Titration

Table of contents

Title	1	Stock solutions	3
FACS - Antibody Titration	2	Preparation of FACS samples	3

1 Stock solutions

- HA Anti Mouse Monoclonal Antibodies in PBS (4.52 μ M)
- HA Anti Mouse Monoclonal Antibodies in PBS (1 μ M)
- HA Anti Mouse Monoclonal Antibodies in PBS (200 nM)
- DBCO-PEG₄-5/6-TAMRA (5 mM)
- Buffers: PBS (DBCO) or PBS-0.1%BSA (antibody)

2 Preparation of FACS samples

- Prepare following tubes:

Tube	[DBCO]	Cells (10 ⁹)	DBCO volume to add (μL)		DBCO/tag ratio
			5 mM		
1	0	200 μL			
2	30 μM	200 μL	1.21		182.2

- React DBCO tubes for 1h in shaking block at 4°C and 500 rpm
- Prepare FACS samples:
 - Spin down the cells for 5 min at 13,400 rpm
 - Discard the supernatant and resuspend with 1 mL ice cold PBS
 - Spin down the cells for 5 min at 13,400 rpm
 - Discard the supernatant and put the pellets on ice until FACS
 - Right before FACS: resuspend with 200 μ L ice cold PBS

- Prepare following tubes:

Tube	[Ab]	PBS-0.1%BSA	Cells (10^7)	Antibody volume to add (μ L)			Ab/tag ratio
				4.52 μ M	1 μ M	200 nM	
1		150 μ L	50 μ L				
2	1 μ M	50 μ L	50 μ L	28.41			1546.6
3	316 nM	150 μ L	50 μ L	15.03			818.2
4	100 nM	150 μ L	50 μ L	4.53			246.6
5	31.6 nM	500 μ L	50 μ L		17.95		216.3
6	10 nM	500 μ L	50 μ L		5.56		67.0
7	3.16 nM	1 mL	50 μ L			16.87	40.6
8	1 nM	1 mL	50 μ L			5.28	12.7
9	316 pM	10 mL	50 μ L			15.92	38.3
10	100 pM	10 mL	50 μ L			5.03	12.1
11	31.6 pM	40 mL	50 μ L			6.33	15.2
12	10 pM	40 mL	50 μ L			2.00	4.8

- Prepare FACS samples:
 - Spin down the cells for 5 min at 13,400 rpm
 - Discard the supernatant and resuspend with 1 mL ice cold PBS-0.1%BSA
 - Spin down the cells for 5 min at 13,400 rpm
 - Discard the supernatant and put the pellets on ice until FACS
 - Right before FACS: resuspend with 200 μ L ice cold PBS-0.1%BSA